Diagnosis of Influenza from Lower Respiratory Tract Sampling after Negative Upper Respiratory Tract Sampling

Virulence 4:1, 82–84; January 1, 2013; © 2013 Landes Bioscience

Diagnosis of influenza from lower respiratory tract sampling after negative upper respiratory tract sampling

Isaac I. Bogoch,[1,2,3,*] Jason R. Andrews,[2,3] Kimon C. Zachary[2,3] and Elizabeth L. Hohmann[2,3]

[1]Divisions of Internal Medicine and Infectious Diseases; Toronto General Hospital; Toronto, ON Canada; [2]Division of Infectious Diseases; Massachusetts General Hospital; Boston, MA USA; [3]Harvard Medical School; Boston, MA USA

Keywords: influenza, human, H1N1 virus, bronchoalveolar lavage, polymerase chain reaction

In this retrospective cohort study, we demonstrate that PCR-confirmed diagnoses of influenza were made solely by lower respiratory sampling in 6.9% of cases, as traditional upper respiratory tract tests were negative, indeterminate or not performed. Clinical features of these cases are presented. Clinicians should consider lower respiratory tract sampling in select cases of influenza-like illness for diagnosis.

Introduction

Clinicians are often faced with the challenge of patients presenting with an influenza-like syndrome despite negative routine influenza investigations. These investigations usually include nasal or nasopharyngeal rapid influenza screens, direct fluorescent antibody (DFA) or PCR tests. Occasionally these diagnostic tests may be falsely negative due to their low sensitivity, as in the case of many rapid-influenza tests,[1] poor technique in specimen collection, delayed transport to the laboratory or the presence of viral inhibitors.[2] Clinicians rely heavily on these investigations as they are readily available and guide therapeutic decisions.

Most influenza infections affect the upper respiratory tract, while lower tract infection typically represents extension from upper airways and may be diagnosed with lower respiratory sampling such as bronchoscopy.[2,3] Occasionally a diagnosis of influenza is missed with upper respiratory tract sampling if pulmonary symptoms are present, and concerns have been raised regarding missing pandemic strain of H1N1[4] and Avian influenza A (H5N1), which have both been shown to infect the lower respiratory tract.[5,6] We present data from our institution where lower respiratory tract sampling aided the diagnosis of influenza and discuss clinical features of these patients.

Methods

The Institutional Review Board at the Massachusetts General Hospital reviewed and approved this study. We performed a retrospective cohort analysis of all cases of PCR-confirmed influenza between December 2009 and April 2011 at the Massachusetts General Hospital [Simplexa™ Influenza A H1N1 (2009), Focus Diagnostics]. We identified all patients where lower respiratory sampling (induced sputum, endotracheal aspiration or bronchoscopy) was used to diagnose influenza. Only cases that were confirmed as PCR-positive were included. Using a standardized data collection form, we recorded patient demographics, influenza diagnostic testing, radiographic features, oxygenation supplementation, clinical features, accompanying comorbid conditions, and outcomes. Obesity was defined by body mass index (BMI) equal to or greater than 30. Patients were defined as immunocompromised if they were taking prednisone (or equivalent) $>$ 15 mg per day for over 2 mo, on active chemotherapy, HIV with CD4 T cell counts less than 200 cells/ml or on other immunomodulatory medications such as biologic therapies like tumor necrosis factor α antagonists.

Results

One hundred and sixteen patients were identified with PCR-confirmed influenza virus between December 2009 and April 2011. Forty-six were typed as pandemic H1N1 and 70 as seasonal influenza A. The average age was 56.6 y (range 1–95) with 60 (51.7%) females. Ninety-four patients (81%) were hospitalized and a total of 6 (5.1%) of died. Sixty-seven (57.8%) had a comorbid condition portending severe influenza. Of these 116 PCR-positive patients, 15 (12.9%) underwent lower respiratory sampling to aid in diagnosis (age range 11–81 y). Ten of these 15 patients (66.7%) were positive for influenza virus in lower respiratory samples. Of these 10, a diagnosis of influenza was made solely by lower respiratory sampling in eight cases (6.9% of total PCR positive cases), as rapid tests, nasopharyngeal DFA or PCR tests were either negative, indeterminate or not performed

*Correspondence to: Isaac I. Bogoch; Email: Isaac.Bogoch@uhn.ca
Submitted: 07/13/12; Revised: 10/03/12; Accepted: 10/05/12
http://dx.doi.org/10.4161/viru.22466

(**Table 1**). Those with positive lower respiratory sampling had an average age of 48.3 y (range 21–81), and were predominantly female (70%). Eight of the 10 patients were receiving oseltamivir at the time of lower respiratory sampling. Seven (70%) patients presented with fever, and the average white blood cell count on presentation was 7.3 cells/ml (range 3.2–16.2) on admission. Radiographic features included 6 (60%) with acute respiratory distress syndrome (ARDS), and 3 (30%) with single or multi lobar consolidative processes. One patient had no obvious radiographic changes from his underlying interstitial lung disease. Eight (80%) patients required care in an ICU and two (20%) patients ultimately died of their illness.

Discussion

Influenza viruses initially infect the upper airways but can directly extend to the lower airways in severe cases, resulting in a viral pneumonia with significant morbidity and mortality.[4] Patients initially present with upper respiratory symptoms, but typically deteriorate from a respiratory standpoint if lower tract symptoms develop, frequently requiring hospitalization or intensive care. Clinicians should be aware that sampling of the upper airways might not be adequate to diagnose these cases. In our series, 6.9% of PCR-documented influenza had negative upper airway sampling, and were diagnosed by either BAL, endotracheal aspiration, or induced sputum. Certain influenza strains such as Avian H5N1 virus[6] are reported to infect lower airways, and case reports suggest that pandemic H1N1 may have a predilection for lower airways as well.[1,5] Indeed, recent studies suggest that mutations to pandemic H1N1 such as D222G/N result in exclusively lower respiratory tract disease.[7] This is concerning as it highlights the possibility for future circulating influenza strains to preferentially infect the lower respiratory tract, and clinicians should be aware that lower-tract sampling may be necessary to make or confirm an influenza diagnosis. It is difficult to ascertain if our case series supports the theory that pandemic H1N1 has a higher probability than seasonal influenza for infecting the lower respiratory tract given the descriptive and retrospective nature; however, 70% of our samples from lower tract cases were confirmed as pandemic H1N1 influenza, yet this only made up 39.7% of all PCR-typed influenza diagnoses.

A weakness of this study is that it is retrospective and the prevalence of lower respiratory tract samples positive for influenza may be underestimated here. Many more patients may have lower respiratory tract disease but were not tested. We also only looked at cases of influenza that were PCR positive, and likely excluded several cases that had negative diagnostic tests. It is not clear why the five patients with lower respiratory tract symptoms who underwent BAL had negative PCR specimens. These may be related to specimen sampling, transportation or processing errors.

Several types of viral pneumonia have been diagnosed via lower respiratory tract sampling. In a primarily immunocompromised cohort of patients with pneumonia, Connoly et al.[8] utilized BAL and culture techniques to diagnose a viral etiology in 615 out of 1,199 specimens. Eleven of these cases were influenza (nine with type A and two with type B). In addition to bronchoscopy, endotracheal aspirates in intubated patients may also be effective in diagnosing lower tract influenza. A small case series from California reported three patients with negative influenza PCR on nasopharyngeal swabs, but positive PCR for pandemic H1N1 influenza with endotracheal aspirates.[9] Two patients in our series were diagnosed by endotracheal aspirate, both of whom had pandemic H1N1 infection. Other helpful diagnostic methods for influenza A include PCR from sputum samples, without the aid of lower respiratory tract sampling. Falsey et al. recently demonstrated the identification of respiratory viruses (primarily influenza A) in 36% of sputum samples tested with PCR compared with 23% of nasopharyngeal samples tested with PCR.[10] Unfortunately, sputum samples are often difficult to obtain, and could only be acquired in 73% of patients.

There are several reasons why results from traditional influenza diagnostic tests may be negative in cases of influenza. Appropriate

Table 1. Characteristics of patients with lower respiratory specimens positive for influenza A (pandemic and seasonal)

Patient	Age (y)	Sex	Rapid test	NP DFA	NP PCR	Comorbidities	Lower respiratory tract sampling	Outcome
			Number in brackets denotes number of specimens					
1	31	F	-(1)	-(1)	n/a	Nil	ETA	Died
2	59	F	n/a	-(2)	n/a	Renal transplant	BAL	Survived
3	21	F	n/a	-(2)	+	Pregnant, obese	BAL	Survived
4	49	F	n/a	+	+	ILD	BAL	Survived
5	54	F	-(1)	-(1)	i(1)	Nil	BAL	Survived
6	26	F	-(2)	-(2)	-(1)	Nil	BAL	Survived
7	73	M	n/a	-(1)	n/a	ILD	BAL	Died
8	37	F	n/a	-(1)	n/a	Nil	ETA	Survived
9	81	M	n/a	-(1)	n/a	Nil	BAL	Survived
10	52	M	-(1)	-(1)	-(1)	HIV, asthma	IS	Survived

NP, nasopharyngeal; DFA, direct fluorescent antibody; i, indeterminate; BAL, bronchoalveolar lavage; ETA, endotracheal aspirate; IS, induced sputum; ILD, interstitial lung disease

nasopharyngeal sampling techniques must be employed, and such tests may ultimately be negative due to inadequate specimen collection. Other possibilities include low levels of viral shedding in the nasopharynx at the time of sampling as the infection has progressed to the lower respiratory tract.[2] Animal models demonstrate more viral replication in the trachea, bronchi and bronchioles with pandemic H1N1 compared with seasonal H1N1, which is restricted primarily to the nasopharynx.[11] Lastly, those with risk factors for severe influenza such as obesity, an immunocompromised state, asthma or pregnancy[12] may be at greater risk of lower respiratory tract involvement and a poor prognosis. Future prospective studies should assess diagnostic characteristics of influenza in relation to the time of sample collection, risk factors for severe disease and clinical disease progression.

Traditional nasopharyngeal diagnostic techniques may miss cases of influenza affecting the lower respiratory tract. Clinicians should have a high degree of suspicion in patients with lower-tract symptoms and a syndrome compatible with influenza, particularly in the setting of pregnancy, obesity or in immunocompromised states. Empiric antiviral therapy is often warranted[13] and sampling of the lower tract by bronchoscopy, endotracheal aspirate or induced sputum may yield a diagnosis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Faix DJ, Sherman SS, Waterman SH. Rapid-test sensitivity for novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med 2009; 361:728-9; PMID:19564634; http://dx.doi.org/10.1056/NEJMc0904264
2. Singh K, Vasoo S, Stevens J, Schreckenberger P, Trenholme G. Pitfalls in diagnosis of pandemic (novel) A/H1N1 2009 influenza. J Clin Microbiol 2010; 48: 1501-3; PMID:20164266; http://dx.doi.org/10.1128/JCM.02483-09
3. Centers for Disease Control and Prevention (CDC). Swine influenza A (H1N1) infection in two children–Southern California, March-April 2009. MMWR Morb Mortal Wkly Rep 2009; 58:400-2; PMID: 19390508
4. Kuiken T, Taubenberger JK. Pathology of human influenza revisited. Vaccine 2008; 26(Suppl 4):D59-66; PMID:19230162; http://dx.doi.org/10.1016/j.vaccine.2008.07.025
5. Yeh E, Luo RF, Dyner L, Hong DK, Banaei N, Baron EJ, et al. Preferential lower respiratory tract infection in swine-origin 2009 A(H1N1) influenza. Clin Infect Dis 2010; 50:391-4; PMID:20047483; http://dx.doi.org/10.1086/649875
6. Beigel JH, Farrar J, Han AM, Hayden FG, Hyer R, de Jong MD, et al. Writing Committee of the World Health Organization (WHO) Consultation on Human Influenza A/H5. Avian influenza A (H5N1) infection in humans. N Engl J Med 2005; 353:1374-85; PMID: 16192482; http://dx.doi.org/10.1056/NEJMra052211
7. Piralla A, Pariani E, Rovida F, Campanini G, Muzzi A, Emmi V, et al. Severe Influenza A Task Force. Segregation of virulent influenza A(H1N1) variants in the lower respiratory tract of critically ill patients during the 2010-2011 seasonal epidemic. PLoS One 2011; 6: e28332; PMID:22194826; http://dx.doi.org/10.1371/journal.pone.0028332
8. Connolly MG, Jr., Baughman RP, Dohn MN, Linnemann CC, Jr.. Recovery of viruses other than cytomegalovirus from bronchoalveolar lavage fluid. Chest 1994; 105:1775-81; PMID:8205876; http://dx.doi.org/10.1378/chest.105.6.1775
9. Chen W, Ayala E, Hill S, Chung J, Miyai T, Kagawa FT, et al. Diagnosis Of 2009 Influenza A H1N1: Diagnostic Utility Of Blind Endotracheal Aspirate In Intubated Patients With False Negative Realtime Reverse Transcriptase Polymerase Chain Reaction Assays From Nasopharyngeal Samples. Am J Respir Crit Care Med 2010; 181:A2623.
10. Falsey AR, Formica MA, Walsh EE. Yield of sputum for viral detection by reverse transcriptase PCR in adults hospitalized with respiratory illness. J Clin Microbiol 2012; 50:21-4; PMID:22090400; http://dx.doi.org/10.1128/JCM.05841-11
11. Munster VJ, de Wit E, van den Brand JMA, Herfst S, Schrauwen EJA, Bestebroer TM, et al. Pathogenesis and transmission of swine-origin 2009 A(H1N1) influenza virus in ferrets. Science 2009; 325:481-3; PMID:19574348
12. Van Kerkhove MD, Vandemaele KA, Shinde V, Jaramillo-Gutierrez G, Koukounari A, Donnelly CA, et al. WHO Working Group for Risk Factors for Severe H1N1pdm Infection. Risk factors for severe outcomes following 2009 influenza A (H1N1) infection: a global pooled analysis. PLoS Med 2011; 8:e1001053; PMID:21750667; http://dx.doi.org/10.1371/journal.pmed.1001053
13. Harper SA, Bradley JS, Englund JA, File TM, Gravenstein S, Hayden FG, et al. Expert Panel of the Infectious Diseases Society of America. Seasonal influenza in adults and children–diagnosis, treatment, chemoprophylaxis, and institutional outbreak management: clinical practice guidelines of the Infectious Diseases Society of America. Clin Infect Dis 2009; 48:1003-32; PMID:19281331; http://dx.doi.org/10.1086/598513